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Induced Changes in Solvent Structure by Phospholipid Monolayer Formation at a Liquid:Liquid Interface

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Abstract

Vibrational sum frequency spectroscopy has been used in conjunction with dynamic surface tension measurements to study formation of a 1,2-dilauroyl-s*n*-phosphatidylcholine (DLPC) monolayer at a water:carbon tetrachloride interface. Surface tension measurements show that an aqueous solution of liquid crystalline phosphocholine vesicles (4.5 μMolar DLPC) requires several hours to form a tightly packed, fully equilibrated monolayer of DLPC monomers. Vibrational spectra of the interfacial region at different stages in the monolayer formation process indicate that the solvent environment undergoes dramatic changes as the monolayer forms. Adsorption of the initial DLPC monomers severely disrupts the interfacial solvent structure. Intensity in the water stretching region oscillates in a systematic fashion during the first two hours of monolayer formation before finally leveling out at an intensity characteristic of the fully equilibrated monolayer. Frequency shifts of the OH stretching vibration show that water molecules with their C_2 axes aligned parallel to the interface experience a markedly different environment than those water molecules aligned perpendicular to the interface. This difference is attributed to the effect of the adsorbed, zwitterionic DLPC headgroups which, if aligned parallel to the interface, can stabilize in-plane water molecules.

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Introduction

Surfactant monolayers at liquid surfaces play a central role in many processes including lubrication, emulsification, and detergent action. [1-3] The timescale over which these monolayers form can be quite long. Monolayers formed by adsorption of surfactants from an underlying bulk solution can require minutes, hours or even days to achieve equilibrium. [4] To study the kinetics of monolayer formation a number of different techniques have evolved, most of which measure the time dependent surface tension as a function of temperature, bulk surfactant concentration and ionic strength. [5, 6] While these methods provide valuable information about the rate at which a monolayer forms, they remain thermodynamic in nature, thus unable to shed insight into changes in molecular structure that accompany monolayer formation. In contrast, newly developed spectroscopic techniques can now directly probe how the interfacial molecular environment evolves during the monolayer formation process. [7] In this report we employ vibrational sum frequency spectroscopy (VSFS) to examine the changes in interfacial solvent structure induced by formation of a phospholipid monolayer at a liquid:liquid interface.

Use of second order nonlinear optical spectroscopy (i.e. VSFS, second harmonic generation (SHG)) has blossomed in recent years. [8-12] These techniques provide a means of probing molecular structure at interfaces. Surprisingly, little work has focused on the time dependent structural behavior of interfacial processes. Shen and coworkers reported using second harmonic generation to follow the adsorption of sodium dodecyl napthalene sulfonate (SDNS) to a freshly cleaned air:water interface. [7] They found that the SDNS optical data tracked dynamic surface tension data quite closely and concluded that SHG represented an optical, non invasive way in which to monitor adsorption kinetics and to determine final monolayer composition. In addition SHG data also allowed the authors to determine the gross orientation of the adsorbed chromophore. This study marked the first application of interfacially specific, non-linear optical spectroscopy to the area dynamics at a liquid surface. However, the data also highlighted a major drawback

associated with SHG: although this technique can disclose the orientation and surface concentration of adsorbed chromophores, it lacks the ability to provide information about detailed conformational structure and the structure of interfacial solvent molecules. Acquiring this type of data requires not only surface specificity but also the molecular specificity that is found in VSFS spectra. [11] Like SHG, VSFS is a second order, nonlinear optical technique that only samples interfaces between isotropic media. However, spectra acquired by VSFS contain molecularly specific information because they disclose the *vibrational* structure of species in the interfacial region.

This report contains the first detailed look at the time dependent changes in molecular solvent structure as a phospholipid monolayer forms at a liquid:liquid interface. Monitoring the vibrational spectra of the interfacial water molecules as phospholipid monomers adsorb to the boundary formed between water and CCl_4 reveals complex changes in the interfacial structure which can not be inferred solely from dynamic surface tension data. We find that initial adsorption of phospholipid monomers disrupts the hydrogen bonding network of interfacial water molecules, and we observe distinct differences between water structure aligned in the plane of the interface and water structure normal (or out-of-plane) to the interface. A large red shift in the vibrational spectra of in-plane water molecules suggests that the zwitterionic phospholipid headgroups lie parallel to the interface, setting up small, microscopic potential gradients which strengthen the hydrogen bonding in this dimension.

Experimental

The experiments described in this work examine the changes in water structure at an aqueous:carbon tetrachloride interface during formation of a phospholipid monolayer. The phospholipid used in these studies, 1,2-dilauroyl-s*n*-phosphatidylcholine (DLPC, Fig. 1), belongs to a family of saturated, symmetric dialkyl phosphocholines. In aqueous solution these molecules spontaneously aggregate in bilayer structures which, upon sonication

above the bilayer gel-liquid crystalline transition temperature, rearrange to form closed shell, bilayer vesicles. [13] At interfaces these vesicles break apart to form monolayers of phosphocholine monomers, [14-17] although the rate at which these monolayers form depends quite sensitively on the phase of the vesicle bilayer. [16, 18] Vesicles in their gel (or frozen) state form monolayers very slowly, requiring days to establish equilibrium. Once above their transition temperature, however, vesicle bilayers exist in a more fluid, liquid crystalline state. Monomers dissociate from vesicles much more quickly, adsorbing to the interface to form equilibrated monolayers in a matter of hours. With a transition temperature of -1° C, DLPC vesicles used in these ambient experiments are in their fluid-like, liquid crystalline state. At aqueous phosphocholine concentrations of 4.5 μmolar, equilibrated monolayers form in ~6 hours.

Stock solutions of DLPC vesicles are prepared by suspending a given amount of the phospholipid in water buffered to a pH of 7.0 with sodium phosphate (~10 mMolar sodium phosphate). The aqueous solution is then sonicated at room temperature (23° C) until the solution becomes clear. Dynamic light scattering experiments show the DLPC vesicles to be ~100-150 nm in diameter, indicating that they are probably multilamellar in nature. Typical stock solution concentrations range from 0.5 - 1.0 mMolar in phosphocholine concentration.

Dynamic surface tension measurements are carried out with a Pt Wilhelmy plate-microbalance assembly. [6] Experiments begin by monitoring the interfacial tension of the neat aqueous:CCl_4 interface. The aqueous phase consists of nanopure water saturated with CCl_4 and buffered to pH 7.0 with a sodium phosphate solution. After establishing interfacial stability and purity, we make a small volume addition of the DLPC vesicle stock solution to the aqueous phase bringing the aqueous DLPC concentration up to the desired value. Additions usually require ~100 μL dispersed in uniform drops upon the water surface. As the monolayer forms the surface tension drops and after several hours, the surface tension begins to approach an asymptotic limit.

To probe molecular structure at the interface as the monolayer forms, we employ the nonlinear optical technique of vibrational sum frequency spectroscopy in a total internal reflection (TIR) geometry. [19] Given its molecular and interfacial specificity, VSFS has developed into a powerful technique for probing solid:liquid, [8] liquid:air, [10] and liquid:liquid interfaces. [19] The method involves two coherent optical fields - typically one fixed frequency visible and one tunable infrared - converging spatially and temporally at the interface. When the infrared radiation is resonant with an allowed vibrational transition of a molecule at the interface, the two waves interact through the resonant term of the second order nonlinear susceptibility ($\chi^{(2)}$) to create a third optical field equal in energy to the sum of the visible and infrared energies.

A TIR geometry can enhance sensitivity by up to three orders of magnitude due to the creation of an evanescent wave at the interface. [20, 21] In a TIR geometry, the visible and infrared beams pass through the high index medium (CCl_4) and the sum frquency (SF) signal is collected in reflection. Spectra presented in this paper come from one of two polarization conditions. Detecting the s polarized SF signal arising from s polarized visible and p polarized infrared ($s_{sum}s_{vis}p_{ir}$ or ssp) probes vibrational motion having a component normal to the plane of the interface. A spectrum recorded under $s_{sum}p_{vis}s_{ir}$ (sps) conditions samples vibrational motion in the plane of the interface.

The laser system used to acquire the data presented in this report has been described previously. [22] Briefly, a fraction of the 800 nm output from a regeneratively amplified Ti:Sapphire laser (Coherent, Quantronix) pumps a white light generation-optical parametric amplifier assembly to provide a source of tunable infrared radiation (3-6 μJ, 18 cm^{-1} FWHM). The remainder of the 800 nm light is used as the fixed frequency visible field. An important aspect of this system is its 1 kHz repitition rate. By averaging only 50 shots per data point, a spectrum of the region from 2800 - 3650 cm^{-1} can be recorded every 3 minutes. Given that a DLPC monolayer requires hours to form, this time resolution is

more than adequate to investigate the structural changes that accompany adsorption of DLPC monomers at the aqueous:CCl_4 interface.

Results and Discussion

A. Dynamic Surface Tension Measurements

A representative dynamic surface tension measurement shown in Figure 2. In this experiment, addition of stock solution raises the bulk DLPC concentration to 4.5 μMolar. The surface tension drops steeply over the first 30 minutes and after 60 minutes assumes an exponential decay, slowly approaching an equilibrium value of 2 mN/m. The experiment shown in Figure 2 ends with a surface pressure of 42 mN/m which is characteristic of monolayers having molecular concentrations of 1.8×10^{14} molecules/cm^2, or, equivalently, 55 $Å^2$/molecule. [23] (Surface pressure is equal the difference between the surface tension of the neat interface ($\gamma_0 = 44$ mN/m) and the terminal surface pressure of the ternary aqueous:phospholipid:CCl_4 system.) This terminal surface concentration agrees with results from surface pressure measurments of phosphocholine monolayers at other organic:aqueous interfaces. [5, 24, 25]

Several important issues stand out in Figure 2. First, the data show that an equilibrated, tightly packed DLPC monolayer requires several hours to form from a solution of liquid crystalline DLPC vesicles. This behavior is quite general for liquid-crystalline vesicles composed of saturated, symmetric dialkyl phosphocholines. [14, 23] For aqueous solutions containing gel state vesicles, monolayer formation can require days and the equilibrated monolayers are considerably expanded. [23] Both the gel and liquid crystalline situations stand in sharp contrast to formation of a monolayer from a solution of simple, soluble surfactants (e.g. sodium dodecyl sulfate, SDS). A similar procedure of starting with a neat aqeuous:CCl_4 interface and then making a small volume addition of SDS stock solution, leads to formation of a complete monolayer in approximately 30 minutes. [19]

Second, during the formation of a phosphocholine monolayer, the surface tension decays smoothly over time. Contained in the data is valuable information about the rate at which the monolayer forms, and future work will show how the kinetics of monolayer formation may be described by a simple first order kinetic model. [18] Absent in this macroscopic analysis, however, are the details about how the molecular structure of the monolayer and the surrounding solvent evolves during monolayer formation. Experiments discussed in this paper provide evidence of complex changes in interfacial solvent environment which can not be inferred from the dynamic surface tension data.

B. Molecular Structure of the Neat Interface and Full Monolayer

A dynamic VSFS experiment consists of first establishing a neat aqueous:CCl_4 interface in a cylidrical quartz cell and recording a VSF spectrum to establish interfacial purity. Next a small volume addition of DLPC stock solution is made to the aqueous phase and a spectrum is recorded every few minutes. Shown in Figure 3 are two spectra taken at the times marked on the dynamic surface tension experiment depicted in Figure 2. The upper panel (Fig. 3a) contains an ssp spectrum of the neat aqueous:CCl_4 interface while the bottom panel (Fig. 3b) depicts an ssp spectrum of an equilibrated, tightly packed DLPC monolayer.

Prior to begining a dynamic VSFS experiment, we record a spectrum of the neat aqueous:CCl_4 interface (Fig. 3a). More than 90% of the total intensity of the spectrum lies under the OH stretching feature centered at 3180 cm^{-1}. Interfacial tension measurements indicate that the boundary between the two solvents is free of contaminants, although we still observe weak intensity in a medium-broad band centered at 2970 cm^{-1}, indicating the presence of trace, surface active impurities. Despite rigorous cleaning procedures and perfect wetting behavior on the quartz cell, this broad, weak feature always appears in the CH stretching region of the nominally neat interface. The concentration of this impurity lies below the detectable limits of various diagnostic techniques (FTIR, NMR, UV-VIS)

which were used to assess the purity of the solvents employed in these experiments. Assuming these limits to lie in the nanomolar (or ppb) range and taking into account the uncertainty (in accuracy) associated with measuring the tension of the neat interface ($\pm$ 0.5 mN/m), we use the Gibbs equation to estimate a contaminant surface concentration of ~1 x 10^{11} molecules/cm^2 or ~0.001 monolayers. We conclude that such low surface concentrations of trace contaminants will not perturb our measurements. That such impurities show up at all reflects the extreme sensitivity of the VSFS-TIR experiments carried out with the optical system described above.

Obvious differences exist between the spectra of the neat interface (Fig. 3a) and the interface with the equilibrated, tightly packed DLPC monolayer (Fig. 3b). In the spectrum of the full monolayer, a number of features assigned to vibrations of the DLPC alkyl tails appear below 3000 cm^{-1}. Most of the structure above 3100 cm^{-1} arises from OH stretching of the interfacial water molecules. This report considers only how the water structure evolves during DLPC monolayer formation at the aqueous:CCl_4 interface. To interpret the spectroscopic changes in water vibrational structure which accompany DLPC adsorption to the interface, we briefly summarize the results of infrared, Raman and VSFS studies of water structure at interfaces.

The topic of water structure at interfaces has received considerable attention in recent years. [26-30] VSF spectra of water at interfaces show up to three distinct features. Based on the results of earlier Raman and infrared studies of bulk water, [31-33] these VSF bands have been assigned to vibrational modes of water molecules in three different environments. A broad band at 3200 cm^{-1} (> 200 cm^{-1} FWHM) has been assigned to the in-phase symmetric stretching modes of coupled water molecules in a symmetric, hydrogen-bonded environment (OH ss-s). [31] This feature indicates a high degree of hydrogen bond ordering such as that found in vibrational spectra of bulk ice. [32] The OH ss-s band appears in VSF spectra of water at both air:water and water:organic interfaces.

A second broad band (OH ss-a) sometimes appears at 3450 cm^{-1} (~200 cm^{-1} FWHM) in the VSF spectra of aqueous surfaces. The exact assignment of this feature is uncertain. Some researchers attribute the band to the symmetric stretch of coupled water molecules in an asymmetric hydrogen bonded environment [31] while others claim that the feature arises from OH stretches of water molecules having bifurcated hydrogen bonds. [33] Regardless, spectral intensity in the 3450 cm^{-1} region suggests greater disorder in the intermolecular hydrogen bonding and a correspondingly more "liquid-like" environment. Although prominent in VSF spectra of the air:water interface, [26, 29] the OH ss-a is frequently absent in spectra of liquid:liquid interfaces, [27] implying that the organic phase imposes a high degree of "ice-like" ordering upon the water molecules at the interface.

The third feature in VSF spectra of water at interfaces appears at 3680 cm^{-1} and is much sharper (60 cm^{-1} FWHM) than the other two vibrational bands. [30] This narrow peak results from "free" or "dangling" OH groups at the interface, water molecules that are missing one or both of their hydrogen bonds. This transition is optically inaccessible in our current experimental configuration.

VSFS spectra of the water:carbon tetrachloride interface at different stages of DLPC monolayer formation show the OH ss-s to be the dominant feature in the water stretching region. The uncertainty associated with fitting such a broad feature (> 200 cm^{-1} FWHM) prevents us from concluding that the OH ss-a band is entirely absent, but if the OH ss-a stretch does appear, it does so with less than 10% of the total integrated OH intensity. Consequently, the analysis that follows will focus exclusively on the intensity and frequency fluctuations in the OH ss-s band which accompany formation of the DLPC monolayer. Complicating the analysis of the OH ss-s band in ssp spectra is the appearance of a medium broad band at ~3100 cm^{-1} (Fig. 3b). Based on Raman measurements [34] as well as selective deuteration studies carried out in this lab, we assign this feature to the asymmetric stretch of methyl groups attached to the quarternary ammonium cation of the

DLPC headgroup (Fig. 1). Data reported below for the OH ss-s band in ssp spectra have been deconvoluted from contributions of the quaternary ammonium methyl asymmetric stretch. In sps spectra this asymmetric methyl vibration never acquires appreciable intensity.

C. Temporal Dependence of OH ss-s Intensity

An experiment begins with the addition of a small volume of DLPC stock solution to initiate monolayer formation. Radical changes in the interfacial water environment immediately ensue. Figure 4 depicts the time dependence of the OH ss-s in both the in-plane (Fig. 4a) and out-of-plane (Fig. 4b) dimensions. Immediately following introduction of the DLPC stock solution, the OH ss-s intensity drops by almost a factor of 10 in-plane and to almost zero out-of-plane. Changes in the OH ss-s intensity can either reflect changes in the number of water molecules contributing to the VSF spectrum or a reorientation of water molecules from an in-plane to an out-of-plane alignment. Based on the correlation observed between the in-plane and out-of-plane intensity, we conclude that the observed time dependent intensity oscillations result from changes in the interfacial depth, rather than net reorientation of the interfacial water molecules from an in-plane to an out-of-plane alignment (or vice versa).

The early time behavior of the OH ss-s band implies that the initial adsorption of phosphocholine monomers reduces the number of contributing OH oscillators. Prior to adsorption, the surface potential (130 mV, calculated [35]) creates a weak double layer in the aqueous phase due to the presence of buffer ions. Using this calculated surface potential in a Gouy-Chapman model of interfacial structure predicts a Debye-Hückel screening length of 3 nm. [27] This screening length provides an appproximate measure of the depth over which the surface potential can influence water structure. Consequently, before a monolayer forms we expect the first ~15 water layers to exhibit surface potential induced anisotropy. As the phosphocholine monomers adsorb, their zwitterionic

headgroups can mitigate the potential drop across the interface, effectively destroying any double layer structure in the aqueous phase. The net result is a reduction in the width of interfacially imposed polar ordering of the aqueous solvent structure and a corresponding decrease in the number of contributing oscillators to the observed spectral intensity.

Between ~15 min and two hours the intensity in both in-plane and out-of-plane dimensions oscillates in a complicated but systematic and reproducible fashion. Based on our analysis of intensity fluctuations arising from different functional groups belonging to the adsorbed DLPC monomers, [36] we believe these changes in OH ss-s intensity are due to the solvent response to two-dimensional phase transitions in the DLPC monolayer. [24, 25] Neutron scattering experiments carried out on phosphocholine monolayers adsorbed to the air:water interface find that the extent of headgroup and backbone hydration depends quite sensitively on surface concentration. [37] Expanded monolayers are solvated by the aqueous phase all the way up to the carboxyl groups or to a depth of ~8 Å. The three carbon glycero backbone is spread in an extended, "butterfly" orientation. At higher surface concentrations, the glycero backbone appears to undergo a drastic conformational change becoming more compact. This restructuring "lifts" the carboxyl groups out of the aqueous phase and leads to a hydration depth of only ~4 Å. In both conformations (expanded and condensed) the zwitterionic headgroup remains roughly parallel to the interface in agreement with theoretical predictions. [38] We believe that in our experiments the oscillations we observe in the OH ss-s intensity arise in response to the structural changes of adsorbed phosphocholine monomers as the monolayer passes from an initial, 2-dimensional gaseous state to a final, tightly packed condensed state having a surface concentration of 1.8×10^{14} molecules/cm^2 (55 Å^2/molecule).

Although earlier work demonstrated how vibrational motion in the alkyl stretching region could constructively or destructively interfere with VSF intensity in the water stretching region, we do not believe that such interference is rsponsible for the observed intensity fluctuations in the OH ss-s band. [26] Interaction between CH and OH

vibrational modes arise when a simple soluble, charged surfactant (i.e. sodium dodecyl sulfate or dodecyl ammonium chloride) adsorb to an interface. Together with their counterions, these singly charged surfactants set up a strong potential gradient normal to the interface which orients water molecules to a depth of approximately the Debye screening length. The extent of spectral interference is directly related to the number of contributing oscillators; a stronger gradient over a longer distance necessarily leads to greater interaction between the CH and OH vibrational modes. In the absence of a potential gradient normal to the interface (such as when both anionic *and* cationic surfactants are adsorbed), spectra show no evidence of interference in the CH and OH stretching regions. [26] Phosphocholine headgroups carry a zwitterionic charge and spectral data show that they lie parallel to the interface at all surface concentrations in agreement with calculations. [38, 39] This situation with the DLPC monolayer shares many similarities with the one where both anionic and cationic simple surfactants are adsorbed to the interface. Consequently we do not expect the CH and OH vibrational modes to interfere with each other to any significant extent.

Furthermore, interference observed in spectra of simple surfactant monolayers adsorbed to aqueous interfaces results from a constructive or destructive interaction between the induced polarizations arising from different CH and OH vibrational modes. Different polarization choices typically access different CH stretching vibrations, thus if interference effects were present in our data, we would expect to see differences between the in-plane and out-of-plane data. Figure 3 shows that the oscillations in the OH ss-s intensities mirror each other in the in-plane and out-of-plane dimensions, implying that interference with the alkyl stretching region is not playing a role in the observed intensity fluctuations.

After ~2 hours, both in-plane and out-of-plane OH ss-s intensities level out at values consistent with an equilibrated, tightly packed monolayer. The in-plane spectra still carry greater intensity in the OH ss-s band than the out-of-plane spectra, although in-plane

intensity with the fully formed monolayer is a factor of 2 less than at the neat interface. Out-of-plane OH ss-s intensity is only slightly less than at the neat water:CCl_4 interface.

D. Temporal Dependence of OH ss-s Frequency

In addition to exhibiting intensity fluctuations as the monolayer forms, the OH ss-s also shifts in frequency as DLPC monomers adsorb to the interface, indicating that changes in the solvent environment immediately follow the commencement of monolayer formation (Figure 5). The frequency of the OH ss-s band provides a very sensitive measure of the extent of hydrogen bonding. [32, 40, 41] Infrared [31] and VSF [41] studies have shown that stronger hydrogen bonding leads to a lower OH ss-s frequency. This phenomenon can be understood in terms of the effect hydrogen bonding has on the covalent OH bonds in liquid water. Stronger hydrogen bonding leads to a lengthening of the covalent OH bonds and a corresponding red shift in the OH ss-s frequency. Conversely, aqueous systems with weak hydrogen bonding typically show the OH ss-s at higher frequencies.[31]

Before the addition of DLPC, the in-plane OH ss-s appears at 3250 cm^{-1} indicating an absence of extensive hydrogen bonding. (Fig. 5a) Immdiately after the monolayer begins to form, however, this band experiences a sharp, ~100 cm^{-1} shift to lower energy. This result suggests that adsorption of DLPC monomers to the aqueous:CCl_4 interface strengthens hydrogen bonding between water molecules aligned parallel to the interface. Although the in-plane OH ss-s band position changes slightly over time, it remains close to the frequency of 3140 cm^{-1} attained during the first fifteen minutes of monolayer formation.

The out-of-plane frequency abruptly shifts 30 cm^{-1} to higher energy following addition of the DLPC stock solution. (Fig. 5b) This blue shift indicates that adsorption of the first DLPC monomers weakens the out-of-plane hydrogen bonding, consistent with the idea that the initial stages of monolayer formation destroys the out-of-plane double layer established by the surface potential at the neat interface. Unlike the in-plane OH ss-s which

remains at its new frequency, the out-of-plane OH ss-s gradually returns to its original center frequency (3180 cm^{-1}) approximately one hour after the monolayer begins to form. Thus, although adsorption of phosphocholine monomers initally disrupts hydrogen bonding normal to the interface, the hydrogen bonding network gradually reestablishes itself as monolayer formation proceeds. The extent of this out-of-plane hydrogen bonding appears to be quite similar for both the neat interface and the interface with the equilibrated, tightly packed DLPC monolayer based on the center position of the OH ss-s band.

From the intensity and the frequency data, we conclude that the out-of-plane water structure is quite similar both before and after monolayer formation. The intensity and the frequency of the out-of-plane plane OH ss-s are approximately the same at $t = 0$ (pre-monolayer) and $t = \infty$ (equilibrated monolayer). The formation process itself, however, induces large fluctuations in the out-of-plane environment as interfacial water molecules respond to reorientation of adsorbed DLPC monomers brought about by the ever increasing surface concentration.

In contrast to the out-of-plane environment, both intensity and frequency data suggest that the solvent environment parallel to the interface experiences fundamental and irreversible changes during DLPC monolayer formation. For the in-plane dimension, intensity data suggest that more water molecules contribute to the VSF spectrum before monolayer formation than after monolayer formation. Furthermore, those in-plane aligned water molecules experience very strong hydrogen bonding immediately after the monolayer begins to form. Taking these observations one step further, we speculate that the orientation of the phosphocholine headgroups bear responsibility for the observed effects. Headgroups arranged parallel to the interface will have domains of large electrostatic potential between them. These potential gradients can align solvating water molecules in a parallel orientation and considerably stabilize in-plane water hydrogen bonding.

We note that molecular dynamics simulations have extensively examined the structure and dynamics of water molecules between phosphocholine bilayers [42-45]

These studies find that the first 1-2 layers of water molecules align with their transition dipoles normal to the interfacial plane, in contrast to what the VSF data suggests. However, several important differences exist between the systems studied in this work and the systems modeled in the simulations. In the simulations the presence of a second headgroup "sheet" located only Ångstroms away from the first introduces strong effects into the surrounding solvent structure. For the monolayer studies presented above, no such perturbative source is present to influence the water molecules solvating the headgroups. Also, the simulations begin with phosphocholine headgroups packed more closely together (47.4 $Å^2$/molecule) than the tightly packed monolayers formed in the experiments described above (55 $Å^2$/molecule). This difference in monomer areas is enough to allow approximately one water molecule per DLPC monomer to intercalate between the headgroups of the adsorbed species. Assuming the headgroups to lie parallel to the interface, any intercalated water molecules will experience very strong asymmetric forces in the in-plane dimension. This environment would necessarily lead to a strong nonlinear response consistent with experimental data.

Conclusion

The experiments described in this work detail how vibrational sum frequency spectroscopy can monitor the real time dynamics of interfacial solvent structure during the formation of a phospholipid monolayer at a liquid:liquid interface. VSFS spectra represent snapshots of the vibrational structure of molecules in the interfacial region at different stages of the monolayer formation process. Intensity changes and spectral shifts of vibrational bands allow observation of how solvent environment evolves as the monolayer passes through different states on the way to forming a tightly packed, condensed structure.

Dynamic surface tension measurements suggest that monolayer formation proceeds in a well defined, continuous fashion with the surface tension smoothly changing from that

of the neat interface to that of the equilibrated, tightly packed monolayer without any discontinuities. Data from VSFS spectra tell a different story. During the first several hours of monolayer formation - a process which takes ~6 hrs for a 4.5 micromolar DLPC aqueous phase at room temperature - spectra of the OH stretching region show dramatic intensity oscillations both in the plane of the interface and normal to the interface. Initial adsorption of phosphocholine monomers to the aqueous:CCl_4 interface appears to severely disrupt the long range structure of the interfacial water molecules. The oscillations in OH ss-s intensity which occur during the next two hours may be correlated with 2-dimensional phase transitions within the monolayer and the resulting reorientation of the adsorbed monomers. This issue will be addressed in future work. [36]

Frequency shifts in the OH ss-s band also shed light into how the solvent environment changes as the monolayer forms. A dramatic red shift in the OH ss-s vibrational band assigned to those water molecules having their symmetry axes parallel to the interface provides strong evidence that the zwitterionic phosphocholine headgroups lie parallel to the interface setting up microscopic regions of strong potential gradients. These gradients may serve to align and stabilize water molecules positioned between the headgroups. For water molecules oriented normal to the interface, the initial stages of monolayer formation abruptly reduces the extent of hydrogen bonding leading to a blue shift in the out-of-plane OH ss-s frequency. Eventually, the out-of-plane hydrogen bonding network reestablishes itself and the out-of-plane OH ss-s returns to its pre-monolayer frequency.

These experiments demonstrate the feasibility of using interfacially specific, nonlinear optical spectroscopic methods to probe the dynamics of interfacial processes. Furthermore, the results show that molecular reorientation which accompanies adsorption of complex surfactants to interfaces can be quite complicated. We are currently examining in greater detail the spectroscopic changes which accompany phospholipid monolayer

formation at a water:CCl_4 interface. Preliminary results indicate that the slower kinetics leads to dampened spectral intensity fluctuations.

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Figure Captions

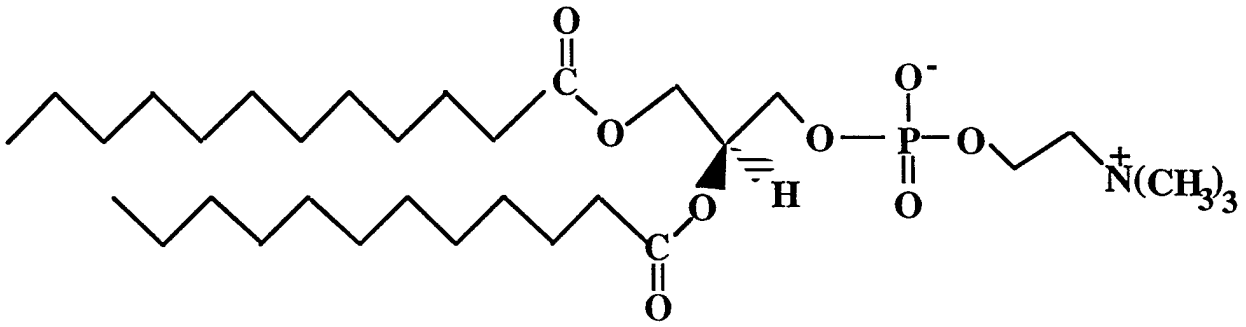
Figure 1. A picture of 1,2-dilauroyl-s*n*-glycero-phosphocholine (DLPC), the phosphocholine used in these studies. DLPC has a zwitterionic headgroup at neutral pH and a pair of saturated, symmetric, C_{12} alkyl chains.

Figure 2. Dynamic surface tension measurement tracking the formation of a DLPC monolayer at the water:carbon tetrachloride interface. Bulk DLPC aqueous concentration is 4.5 μmolar and the temperature is 23° C. After a steep initial descent during the first hour of monolayer formation, the surface tension begins to decay in an exponential fashion, slowly approaching the asymptotic limit characteristic of a tightly packed monolayer. The surface tension of the neat aqueous:carbon tetrachloride interface is 44 mN/m while the tension of the interface with the fully equilibrated, tightly packed monolayer is ~2 mN/m, giving a surface pressure of 42 mN/m.

Figure 3. VSFS spectra of (a) the neat aqueous:carbon tetrachloride interface and (b) the aqueous:carbon tetrachloride interface to which a tightly packed DLPC monolayer has been adsorbed. Spectra were recorded under $s_{sum}s_{vis}p_{ir}$ conditions and intensities have been corrected for power. Spectra were fit using Lorentzian profiles and assignments were based on previous VSFS, IR, and Raman studies (see text).

Figure 4. Time dependent intensity in the OH ss-s band (a) in the plane of the interface ($s_{sum}p_{vis}s_{ir}$) and (b) out of the plane of the interface ($s_{sum}s_{vis}p_{ir}$). Intensities have been corrected for power and for differences in Fresnel coefficients. Error bars reflect a combination of uncertainties in the spectral fitting and experimental reproducibility.

Figure 5. Time dependent center position of the OH ss-s band (a) in the plane of the interface ($s_{sum}p_{vis}s_{ir}$) and (b) out of the plane of the interface ($s_{sum}s_{vis}p_{ir}$). Band positions have been calibrated against a monochrometer and uncertainties reflect a combination of ω_{ir} bandwidth (18 cm^{-1}) and reproducibility of spectral fitting.



1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC, C_{12})

Figure 1
Walker, et al.

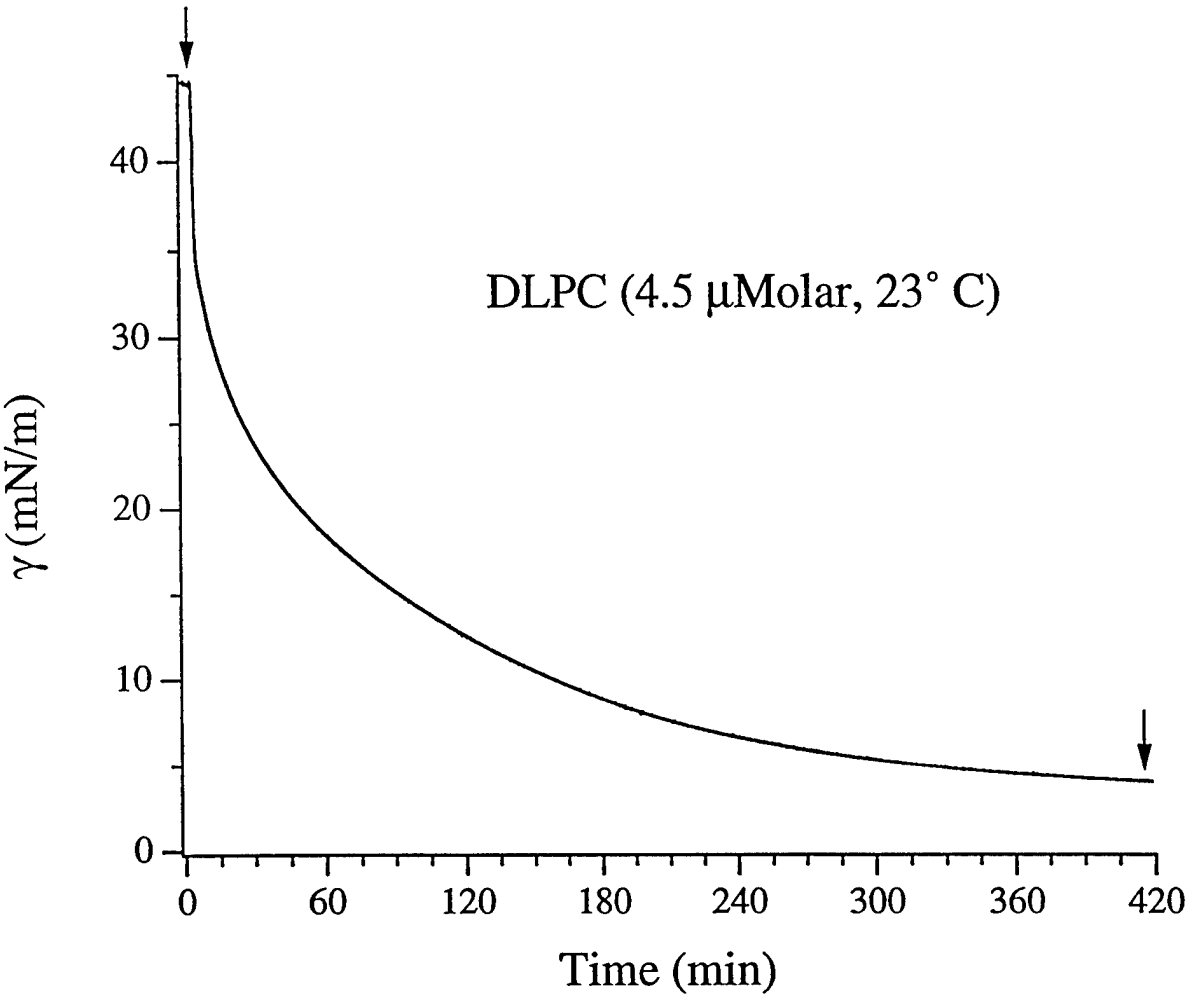


Figure 2
Walker, et al.

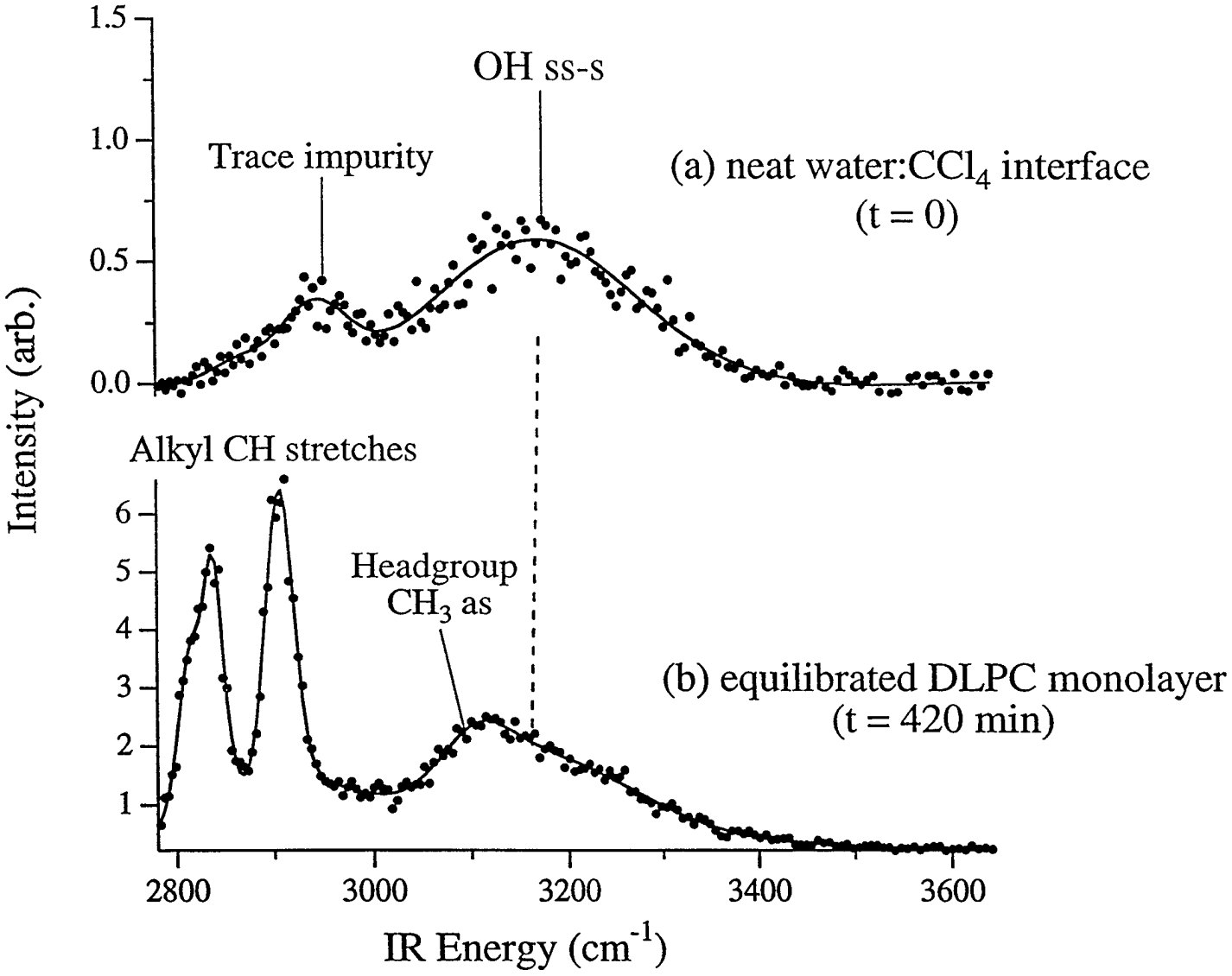


Figure 3
Walker, et al.

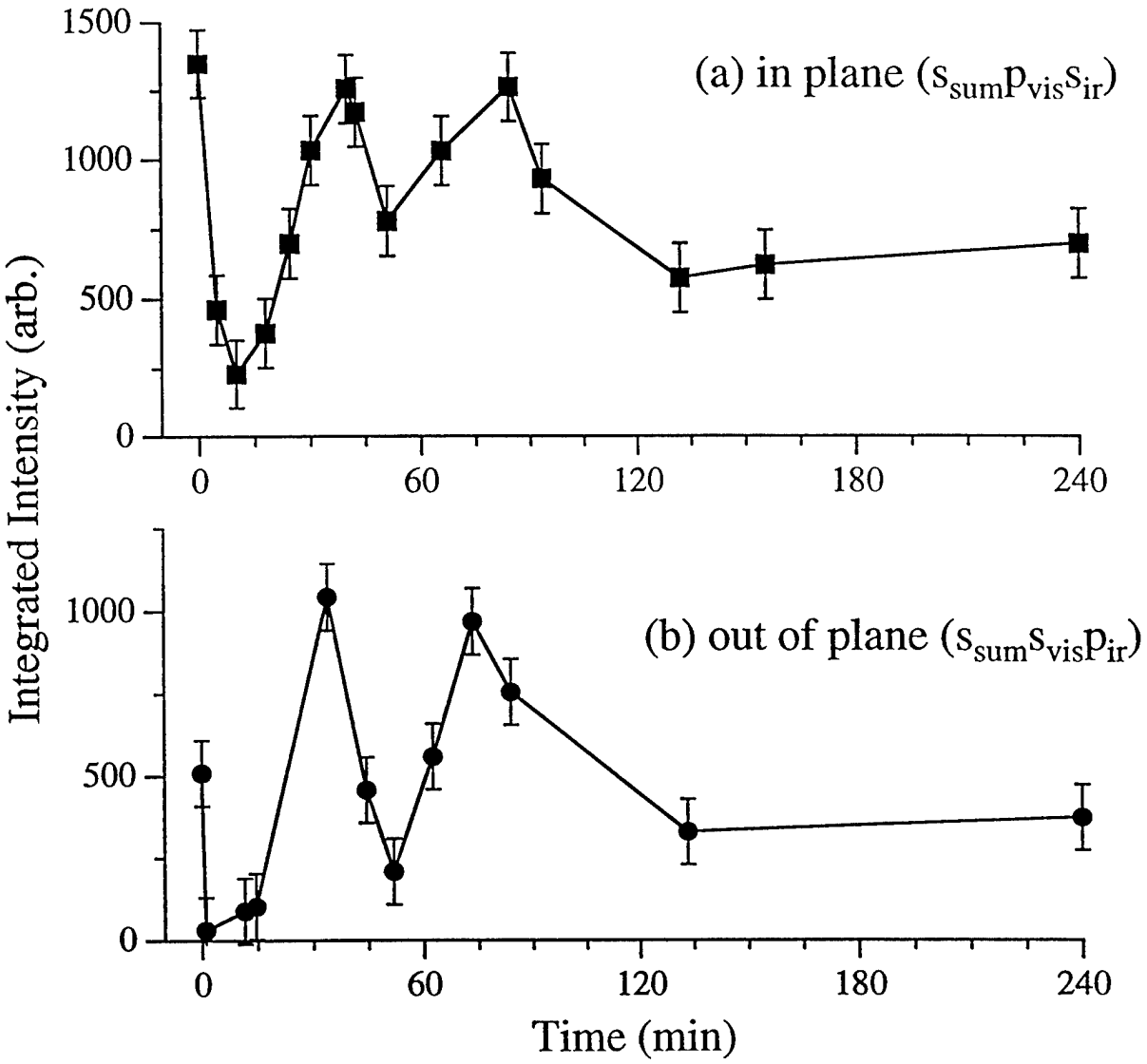


Figure 4
Walker, et al.

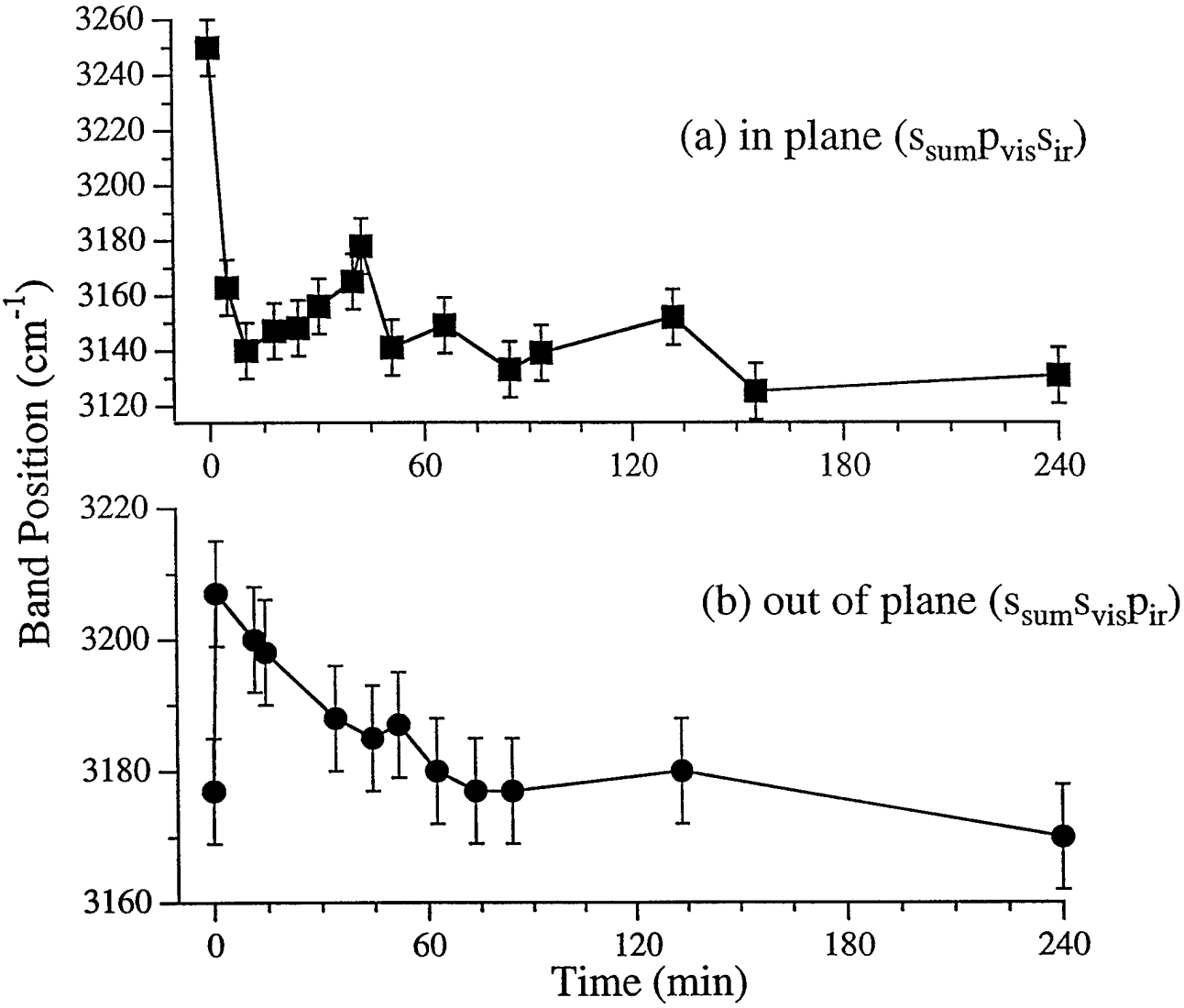


Figure 5
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